Research Article

Total body computed tomography scan in the initial work-up of Binet stage A chronic lymphocytic leukemia patients: results of the prospective, multicenter O-CLL1-GISL study

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Total body computed tomography (TB-CT) scan is not mandatory in the diagnostic/staging algorithm of chronic lymphocytic leukemia (CLL). The aim of this study was to determine the value and prognostic significance of TB-CT scan in early stage CLL patients. Baseline TB-CT scan was performed in 240 Binet stage A CLL patients (179 Rai low- and 61 Rai intermediate-risk) included in a prospective multicenter observational study (clinicaltrial.gov ID:NCT00917549). The cohort included 69 clinical monoclonal B lymphocytosis (cMBLs). Patients were restaged considering only radiological data. Following TB-CT scans, 20% of cases reclassified as radiologic Binet (r-Binet) stage B. r-Binet B patients showed a higher incidence of unfavorable cytogenetic abnormalities ($P = 0.027$), as well as a shorter PFS ($P = 0.001$). At multivariate analysis, r-Binet stage [HR = 2.48; $P = 0.004$] and IGHV mutational status [HR = 3.01; $P = 0.002$] retained an independent predictive value for PFS. Among 179 Rai low-risk cases, 100 were redefined as r-Rai intermediate-risk based upon TB-CT scan data, showing a higher rate of cases with higher ZAP-70 ($P = 0.033$) and CD38 expression ($P = 0.029$) and β2-microglobulin levels ($P < 0.0001$), as well as a shorter PFS than those with r-Rai low-risk ($P = 0.008$). r-Rai stage [HR = 2.78; $P = 0.046$] and IGHV mutational status [HR = 4.25; $P = 0.009$] retained a significant predictive value for PFS at multivariate analysis. Forty-two percent of cMBL patients were reclassified as r-small lymphocytic lymphomas (r-SLLs) by TB-CT scan. TB-CT scan appears to provide relevant information in early stage CLL related to the potential and the timing of patients to progress towards the more advanced disease stages. Am. J. Hematol. 88:539–544, 2013. © 2013 Wiley Periodicals, Inc.

Introduction

Chronic lymphocytic leukemia (CLL) patients show a variable clinical course with some cases having an almost normal progression-free life span and others require therapy shortly after diagnosis [1]. Rai et al. and Binet et al. staging systems, which consider the extent of lymphadenopathy, organomegaly, and cytopenias, are useful for assessing prognosis [2,3]. However, for early stage patients it is virtually impossible to predict the clinical course. The use of serum indicators, i.e., β2-microglobulin [4] and free-light chains [5], along with molecular and cytogenetic markers [6] may provide valuable information, although most of these markers are not routinely employed in clinical practice.

Additional Supporting Information may be found in the online version of this article.

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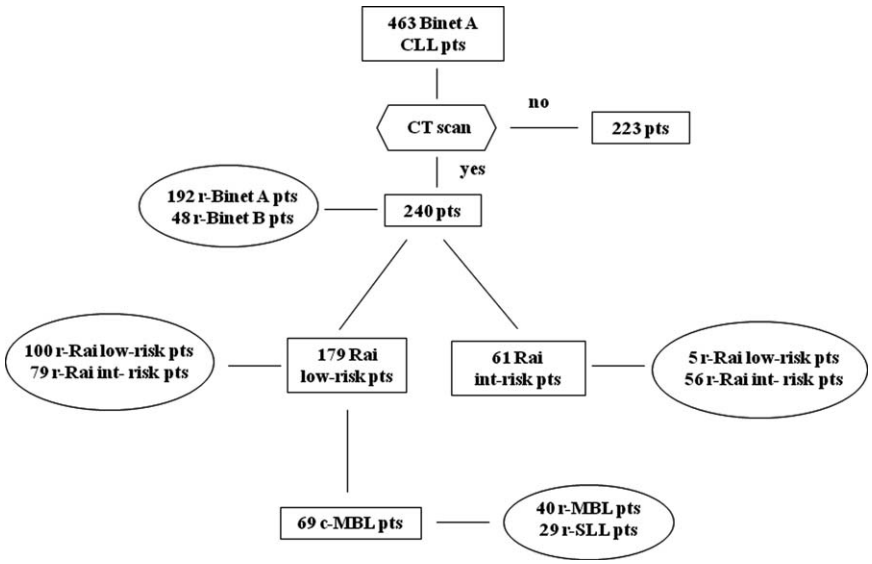

Figure 1. CONSORT diagram of the study. The diagram shows the distribution of CLL cases enrolled in the study according to execution of TB-CT scans and to clinical and radiological staging systems. Int: intermediate; r: radiological.

The current staging systems are based on clinical examination and blood counts, an approach maintained by the National Cancer Institute CLL Working Group (NCI/WG) guidelines, which do not recommend computed tomography (CT) scan at diagnosis [7,8]. For this reason, abdominal and thoracic lymphadenopathies are not incorporated in the staging procedures. Moreover, certain liver or spleen enlargements may go undetected at clinical examination and hence not be computed into the staging determination. This is in contrast with the staging procedure of nearly all other lymphoid malignancies, where CT and often PET scans are highly recommended or mandatory [9,10]. Nonetheless, Muntanola et al. have shown that among Rai 0 patients abdominal lymphadenopathy or splenomegaly detected by CT scan predict disease progression [11]. Despite this, there have been no further studies addressing the issue systematically.

In the present study, we investigated the value of TB-CT scans in a cohort of Binet A patients participating in a prospective multicenter observational study. Subanalyses have also been performed in the setting of Rai low-risk and of cMBL cases, included in this cohort given the particular study design.

Methods

Patients

Newly diagnosed CLL patients from several Italian Institutions were prospectively enrolled into the O-CLL1-GISL protocol (clinicaltrial.gov identifier: NCT00917540). The inclusion criteria for CLL diagnosis, employed at the time of study start, followed the NCI/WG guidelines established in 1996 requiring >5.000 lymphocytes/microliter in the peripheral blood [7]. Exclusion criteria were the following: (i) diagnosis >12 months before registration; (ii) $CD5^-$ and/or $CD23^-$ B-lymphoproliferative disorders; (iii) clinical Binet stage B or C; (iv) need of therapy according to NCI/WG guidelines; and (v) age >70 years. CLL cell phenotype, CD38 and ZAP-70 expression, and *IGHV* mutational status was centralized at the laboratory in Genoa, while cytogenetic analyses in Milan. All patients underwent bone marrow biopsy at baseline. This study was approved by the ethics committees of the participating centers. All patients provided written informed consent.

To date, of 463 Binet stage A patients enrolled 240 (179 Rai low- and 61 Rai intermediate-risk) [12] underwent TB-CT scan at baseline and were included in this study (Fig. 1). Our series is representative of all 463 cases, since there is a similar distribution of clinical and biological parameters in the two subgroups (Supporting Information Table I). Sixty-nine of the 240 cases satisfied the 2008 NCI/WG criteria for the definition of cMBL ($<5.0 \times 10^9$ B lymphocytes/L in the peripheral blood and no apparent lymph node, spleen, or liver enlargement) [8].

All patients underwent follow-up visit every 6 months until progression defined as need for therapy according to NCI/WG guidelines [8]. A minimum follow-up of 6 months was available for 230/240 cases and were evaluated for progression-free survival (PFS).

Assessment of biological markers

Cytogenetic abnormalities involving deletions at chromosomes 11q23, 13q14, and 17p13 and trisomy 12 were evaluated by FISH in purified $CD19^+$ population as previously described [5]. Cytogenetic abnormalities were clustered in that risk groups [i.e., low- (del(13q14) and normal), intermediate- (trisomy 12), and high-risk [del(11q22) and del(17p13)]. CD38-positive leukemic cells were measured by triple staining with CD19-FITC (BD Biosciences), CD38-PE (BD Biosciences), and CD5-PC5 (Beckman Coulter). The cells were analyzed using a FACSCalibur flow-cytometer (BD Biosciences). CD38-positive cases were indicated as those having greater than 30% positive cells. ZAP-70 was determined by flow-cytometry with a ZAP-70-FITC (clone 2F3.2, Millipore) or an isotype control mAb (mouse IgG2a-FITC BD Biosciences) as previously described [6]. ROC curve analysis identified 40% of ZAP-70-positive cells as the best cut-off value for distinguishing *IGHV* unmutated (*IGHV*-UM) from *IGHV* mutated (*IGHV*-M) cases (AUC = 0.64, $P = 0.002$). Therefore, 40% was used as threshold to identify ZAP-70-positive cases. *IGHV* mutational status was assessed on cDNA specimens [13]. Sequences were aligned to the IMGT directory and analyzed using IMGT/VQUEST software. Sequences differing more than 2% from the corresponding germ-line gene were considered *IGHV*-M [13].

Integration of TB-CT scans in the Binet and Rai staging systems

CT scans of the head-neck, chest, abdomen, and pelvis were performed in craniocaudal direction, with a 5- to 8-mm collimation. The scans were routinely acquired 70 s after the injection of 100–200 ml of iodinated contrast material, at a rate of 3 ml/s by powered injection.

Radiologists evaluated TB-CT scans according to the following shared criteria. The assessment of nodal groups status was based on size [14], the maximum short-axis diameter of lymph nodes was taken as the most reliable parameter to determine lymph node size according to standard criteria [15]. Lymph nodes >10 mm in diameter were considered abnormal according to the Binet staging system.3. Furthermore, lymph nodes were also considered abnormal if multiple nodes measuring <10 mm in the short-axis diameter were seen in a single region [16]. Splenomegaly and hepatomegaly were defined based upon the changes that an enlarged spleen and liver caused in relation to the other abdominal viscera; liver and spleen volume were calculated according to standard diagnostic criteria [17]. The normal volume for the spleen was <375 cm^3 for males and <309.55 cm^3 for females, for the liver <2275.47 cm^3 for males and <1927.46 cm^3 for females [17]. Based only on TB-CT scan data, all patients were reclassified

TABLE I. Discrepancies Between Radiological and Clinical Examination for Lymph Node Involvement and Organomegalies

	No. of patients (%)				
	Clinical examination	Radiological examination	Discordance	CT scan+/ Clinical grounds−	CT scan-/ Clinical grounds+
Head-neck adenopathy	31 (12.9)	62 (25.8)	53 (22.1)	42 (17.5)	11 (4.6)
Axillary adenoapathy	28 (11.7)	88 (36.7)	70 (29.2)	65 (27.1)	5 (2.1)
Inguinal adenopathy	1 (0.4)	33 (13.7)	32 (13.3)	32 (13.3)	0 (0)
Splenomegaly	10 (4.1)	35 (14.6)	31 (12.9)	28 (11.7)	3 (1.2)
Hepatomegaly	8 (3.3)	22 (9.2)	24 (10)	19 (7.9)	5 (2.1)

according to Binet classification and assigned to radiologic Binet (r-Binet) A (patients with <3 areas of nodal or organ enlargement) or r-Binet B (patients with >3 areas of nodal or organ enlargement). In this modified restaging, we considered as areas of involvement: (1) head and neck lymph nodes (including Waldeyer's ring) (uni-/bilateral), (2) axillary lymph nodes (uni-/bilateral), (3) inguinal lymph nodes (uni-/bilateral), (4) thoracic lymph nodes (uni-/bilateral), (5) abdominal lymph nodes (uni-/bilateral), (6) splenomegaly, (7) hepatomegaly. Likewise, 179 clinical Rai low-risk patients within this cohort were reclassified as r-Rai low-risk (patients without lymphadenopathies and organomegaly) and r-Rai intermediate-risk groups (patients with lymphadenopathies at any site, and/or splenomegaly and/or hepatomegaly). Finally, 69 cMBL patients were divided into r-cMBL and r-SLL cases. Since, for SLL cases the staging should be carried out according to lymphoma guidelines, only cases with lymph nodes >15 mm in diameter and/or splenomegaly were considered as r-SLL [18,19].

Statistical analysis

SPSS for Windows, v13.0, 2004 software (SPSS, UK) was used for all analyses. For categorical variables, statistical comparisons were performed using two-way tables for the Fisher's exact test and multiway tables for the Pearson's Chi-square test. PFS analyses were performed using the Kaplan–Meier method in patients with a minimum 6 month follow-up. Statistical significance of associations between individual variables and survival was calculated using the log-rank test. Prognostic impact for the outcome variable was investigated by univariate and multiple Cox regression analysis. Data are expressed as hazard ratio (HR) and 95% confidence interval (CI). A value of $P < 0.05$ was considered significant.

Results

Clinical versus radiologic staging

This cohort included 240 Binet A patients (179 Rai low- and 61 Rai intermediate-risk). Of these, 69 met the diagnostic criteria of cMBL. Baseline patient features are listed in (Supporting Information Table II). Median age was 60 years (range, 33–70) and 137 (57.1%) were male; roughly one-third of cases showed elevated β2-microglobulin levels. Seventy-nine patients (33.1%) were *IGHV*-UM, 45.8% were ZAP-70-positive, and 19.2% CD38-positive. FISH data, available in 226/240 cases, identified del(13q14) as the most frequent abnormality followed by trisomy 12, while there was a low incidence of del(11q22.3) cases and an even lower percentage of del(17p13) cases.

Sixty-one out of 240 (25.4%) patients showed clinical lymphadenopathies and/or organomegaly. Thirty-two patients showed involvement of only one lymph node area, 13 of two lymph node areas, 2 of one lymph node area and splenomegaly and 14 splenomegaly and/or hepatomegaly. Thirty-one out of 240 cases (12.9%) showed head and neck lymphadenopathies, 28 (11.7%) axillary lymphadenopathies and 1 (0.4%) inguinal lymphadenopathy. Ten cases (4.2%) had splenomegaly and 8 (3.3%) hepatomegaly (Supporting Information Table II).

At TB-CT scan, 84/240 patients showed no organomegaly or lymphadenopathies; head and neck lymph nodes were enlarged in 62 cases (25.8%), axillary lymph nodes in 88 (36.7%), inguinal lymph nodes in 33 (13.8%), thoracic lymph nodes in 22 (9.2%), and abdominal lymph nodes in 59 (24.6%). Lymphadenopathies had diameters between 15 and 30 mm. Moreover, 35 (14.6%) cases presented splenomegaly, and 22 (9.2%) hepatomegaly (Supporting Information Table II).

There was discordance between radiological and clinical examination in 53/240 (22.1%) cases for head and neck lymph node involvement; in 42 cases, TB-CT scans revealed lymphadenopathies not seen at clinical assessment, while in 11 cases lymphadenopathy was not confirmed radiologically (Table I). There was discordance for axillary lymph node involvement in 70/240 (29.2%) patients; TB-CT scans did not confirm lymphadenopathy in 5 cases, while in 65 cases negative at clinical level radiological examination revealed lymphadenopathy. TB-CT scans demonstrated inguinal lymphadenopathy not observed at physical examination in 32/240 (13.3%) patients. There were discrepancies regarding splenomegaly in 31 (12.9%) cases; the TB-CT scans did not confirm the presence of clinical splenomegaly in three cases, while they revealed splenomegaly not identified on clinical examination in 28 cases. There was discordance regarding hepatomegaly in 24 (10%) cases; TB-CT scan identified 19 cases with hepatomegaly undetected at clinical examination, and did not confirm the presence of clinical hepatomegaly in 5 cases.

Restaging of patients: Binet A cases

Forty-eight out of 240 Binet A patients (20%) were converted into r-Binet stage B. Among these reclassified patients 64.6% were male, 35.9% showed elevated β2-microglobulin level, 41.7% were *IGHV*-UM, 50% had a high ZAP-70 expression, 27.1% were CD38-positive, and 15.9% showed high-risk FISH lesions (Table II). r-Binet B patients showed a statistically higher rate of high-risk cytogenetic cases than r-Binet A patients (15.9% vs. 5.5%; $P = 0.027$). The distribution of the remaining parameters were not statistically significant (Table II).

After a median follow-up of 31 months, 56/230 (24.3%) evaluable cases showed disease progression. r-Binet B patients showed a significantly shorter PFS than those with r-Binet A (2-year PFS probability, 71.2% vs. 87.4%; $P = 0.001$) (Fig. 2). At Cox univariate analysis, r-Binet stage [HR = 2.55, 95%CI (1.38–4.71); $P = 0.003$], CD38 [HR = 3.19, 95%CI (1.8–5.79); $P < 0.0001$] and ZAP-70 expression [HR = 2.29, 95%CI (1.3–4.02); $P = 0.004$] and IGHV mutational status [HR = 4.16, 95%CI (2.37–7.31); $P < 0.0001$] showed a statistically significant impact on PFS (Table III). At Cox multivariate analysis, only r-Binet stage [HR = 2.48, 95%CI (1.33–4.62); $P = 0.004$] and IGHV mutational status [HR = 3.01, 95%CI (1.48–6.15); $P = 0.002$] maintained an independent prognostic impact (Table III).

Moreover, 22 cases with thoracic lymphadenopathies showed a nonstatistically different PFS as compared to 208 cases without pathologic thoracic lymph nodes (2-year PFS probability, 76.5% vs. 85.8%, $P = 0.16$); while 59 cases with abdominal lymphadenopathies had a significantly shorter PFS than 161 cases with a normal abdominal CT scan (2-year PFS probability, 74.4% vs. 88.2%, $P < 0.0001$).

Finally, TB-CT scan allowed the early identification of a second neoplasm in 2 cases (lung cancer and renal cell carcinoma).

TABLE II. Correlation Among Main Initial Characteristics and Staging Systems Integrated with TB-CT Scan

Clinical staging	Binet stage A ($n=240$)			Rai low-risk stage ($n=179$)			cMBL ($n=69$)		
Radiological staging	% of Binet stage A pts ($n=192$)	% of Binet stage B pts ($n=48$)	P	% of Rai low-risk stage pts ($n=79$)	% of Rai intermediate-risk stage pts ($n=100$)	P	% of cMBL pts ($n=40$)	% of SLL pts ($n=29$)	P
Sex Male/Female	55.2/44.8	64.6/35.4	ns	46.8/53.2	60/40	ns	47.5/52.5	51.7/48.3	ns
Age <60/>60 yr	46.4/53.6	56.3/43.7	ns	45.6/54.4	49/51	ns	45/55	37.9/62.1	ns
Lymphocyte count $<30/>30 \times 10^9$/l	90.6/9.4	93.8/6.2	ns	97.5/2.5	90/10	ns	–	–	–
β2-microglobulin ($n=203$) normal/elevated	75/25	64.1/35.9	ns	88.9/11.1	61.8/32.2	<0.0001	83.9/16.1	62.5/37.5	ns
LDH ($n=216$) normal/elevated	92.5/7.5	95.3/4.7	ns	95.6/4.4	91.6/8.4	ns	91.7/8.3	92.6/7.4	ns
CD38 expression negative/positive	82.8/17.2	72.9/27.1	ns	89.9/10.1	77/23	0.029	90/10	75.9/24.1	ns
ZAP-70 expression ($n=238$) negative/positive	55.3/44.7	50/50	ns	64.1/35.9	47.5/52.5	0.033	62.5/37.5	44.8/55.2	ns
IGHV mutational status ($n=239$) mutated/germline	69.1/30.9	58.3/41.7	ns	74.4/25.6	66/34	ns	82.1/17.9	62.1/37.9	ns
Cytogenetic risk ($n=226$) low + intermediate/high	94.5/5.5	84.1/15.9	0.027	94.4/5.6	92.9/7.1	ns	97.1/2.9	92.9/7.1	ns

ns, not significant.

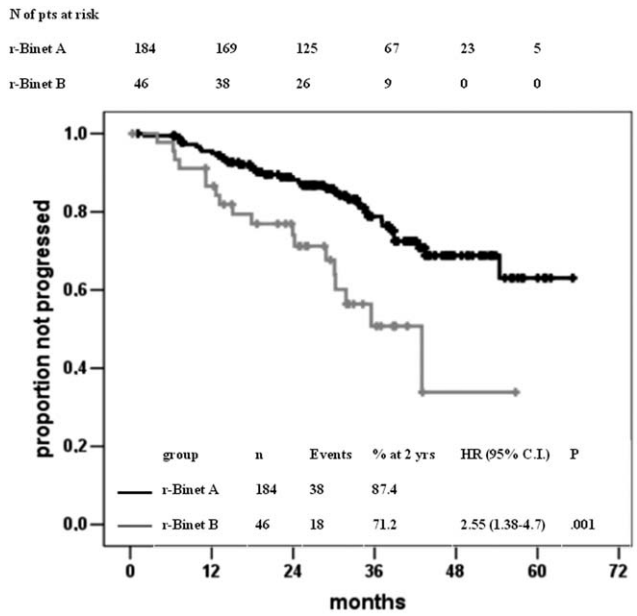

Figure 2. PFS according r-Binet stage. Taking into consideration only radiological data 48/240 Binet A patients (20%) were converted into r-Binet stage B. r-Binet B patients showed a significantly shorter time to progression than those with r-Binet A (2-year PFS probability, 71.2% vs. 87.4%; $P=0.001$).

Restaging of patients: Rai low-risk cases

There were 179 Rai low-risk and 61 Rai intermediate-risk cases among Binet A patients. On the basis of CT scan data, 100/179 (55.9%) low-risk patients were redefined as r-Rai intermediate-risk. This subset of patients showed a statistically higher rate of cases with higher ZAP-70 (52.5% vs. 35.9%; $P=0.033$) and CD38 expression (23% vs. 10.1%; $P=0.029$) and β2-microglobulin levels (32.1% vs. 11.1%; $P<0.0001$) than r-Rai low-risk patients (Table II). After a median follow-up of 30 months, 29/172 (16.9%) evaluable cases showed disease progression. r-Rai intermediate-risk patients had a significantly shorter PFS than r-Rai low-risk patients (2-year PFS probability, 85% vs. 97.2%; $P=0.008$) (Fig. 3). At Cox univariate analysis, r-Rai stage [HR = 3.86, 95%CI (1.46–10.26); $P=0.007$], β2-microglobulin level [HR = 3.15, 95%CI (1.41–7.04); $P=0.005$], CD38 [HR = 4.14, 95%CI (1.87–9.14); $P=0.001$], ZAP-70 expression [HR = 2.57, 95%CI (1.16–5.68); $P=0.02$] and IGHV mutational status [HR = 5.43, 95%CI (2.42–12.2); $P<0.0001$] showed a statistically significant impact on PFS (Table III). At Cox multivariate analysis, only r-Rai stage [HR = 2.78, 95%CI (1.02–7.59); $P=0.046$] and IGHV mutational status [HR = 4.25, 95%CI (1.43–12.6); $P=0.009$] maintained an independent prognostic impact (Table III).

Furthermore, thoracic CT scans failed to significantly separate the clinical outcome of those 13 cases with thoracic lymphadenopathies from 159 radiologically negative cases (2-year PFS probability, 90.4% vs. 90.9%, $P=0.2$), while 34 cases with abdominal lymphadenopathies showed a statistically shorter PFS than 138 cases without pathological abdominal lymph nodes (2-years PFS probability, 82.5% vs. 92.3%, $P<0.0001$).

Restaging of patients: cMBL cases

On the basis of TB-CT scan data, 29/69 (42%) cMBL patients were redefined as r-SLLs. Of note, no statistically different distribution of clinical and biological parameters was observed between r-cMBL and r-SLL patients (Table II). After a median follow-up of 35 months, 5/66 cases evaluable presented progression (3 r-cMBLs and 2 r-SLLs). Considering all clinical Rai low-risk patients, no statistical difference in PFS was observed between those reclassified as r-cMBL, r-SLL, or r-Rai low-risk patients, while r-Rai intermediate-risk cases showed a statistically shorter PFS (2-years PFS probability, r-cMBL 97%, r-SLL 92.3%, r-Rai low-risk 97.2%, r-Rai intermediate-risk 81.9%, $P<0.0001$).

Discussion

CT scan is not routinely recommended for CLL patient work-up in contrast with other low-grade lymphoproliferative disorders [20]. Moreover, the studies investigating the clinical significance of TB-CT scan in CLL are scanty.

Herein, we investigated whether early stage CLL can be upstaged using TB-CT scan and whether these upstaged patients have clinical and biological characteristics and PFS (determined by need for therapy) differing from those who are not upstaged. A similar evaluation was performed in cMBL patients. This category of patients was included in the O-CLL1 protocol when enrollment began in 2006 since the investigators followed the 1996 NCI/WG guidelines for CLL diagnosis [7]. cMBL was later recognized as a separate entity by 2008 NCI/WG guidelines [8]. Finally, we evaluated the prognostic impact of thoracic and abdominal lymphadenopathies.

In our series, 25% of 240 Binet A patients presented clinical lymphadenopathy and/or organomegaly; this percentage reached 65% following TB-CT scan, mainly due to the identification of thoracic and abdominal lymphadenopathies in 9% and in 24% of the cases, respectively. Studies

TABLE III. Univariate and Multivariate Analyses (Cox Model) of Progression-Free Survival

	Clinical Binet stage A patients (n = 230)							Clinical Rai low-risk patients (n = 172)						
		Univariate analysis			Multivariate analysis				Univariate analysis			Multivariate analysis		
	No of pts (No of events)	HR	95% CI	*P*	HR	95% CI	*P*	No of pts (No of events)	HR	95% CI	*P*	HR	95% CI	*P*
r-Binet stage A/B	184/46 (38/18)	2.55	1.38–4.71	0.003	2.48	1.33–4.62	0.004	–	–	–	–	–	–	–
r-Rai stage low-/intermediate-risk	–	–	–	–	–	–	–	77/95 (7/22)	3.86	1.46–10.26	0.007	2.78	1.02–7.59	0.046
CD38 expression negative/positive	187/43 (34/22)	3.19	1.8–5.79	<0.0001	1.54	0.79–3.02	0.2	143/29 (16/13)	4.14	1.87–9.14	0.001	1.63	0.67–3.95	0.279
ZAP-70 expression negative/positive	124/105 (22/34)	2.29	1.3–4.02	0.004	1.25	0.64–2.44	0.51	94/77 (10/19)	2.57	1.16–5.68	0.02	0.81	0.3–2.19	0.68
IgVH mutational status mutated/germline	154/75 (21/35)	4.16	2.37–7.31	<0.0001	3.01	1.48–6.15	0.002	120/51 (9/20)	5.43	2.42–12.2	<0.0001	4.25	1.43–12.6	0.009
Cytogenetic risk low + intermediate/high	202/16 (48/7)	1.76	0.75–4.13	0.19	–	–	–	153/10 (26/3)	1.57	0.47-5.23	0.46	–	–	–

Figure 3. PFS according r-Rai stage in clinical Rai stage 0 patients. Based upon CT scan data, 100/179 (55.9%) Rai low-risk patients were redefined as r-Rai intermediate-risk. r-Rai intermediate-risk patients had a significant shorter time to progression than those with r-Rai low-risk (2-year PFS probability, 85% vs. 97.2%; $P = 0.008$).

investigating thoracic lymph node involvement in a large cohort of CLLs are unavailable, although thoracic lymphadenopathies are traditionally considered infrequent [21]. We found a more common involvement of abdominal lymph nodes at a rate comparable with that reported by Muntanola et al., who demonstrated abdominal lymphadenopathies in 27% of 140 Rai 0 cases by CT scans [11]. Moreover, we observed an important discrepancy between radiological and clinical examinations concerning superficial lymphadenopathies (see Table II), possibly due to the relatively small lymph node diameter in numerous patients. Moreover, spleno-/hepatomegaly were more easily detected radiologically. Together the findings indicate a greater sensitivity of the imaging techniques. Does this greater sensitivity translate into clinically relevant information? Indeed, PFS was shorter for r-Binet B than for r-Binet A patients. Interestingly, multivariate analysis demonstrated that *IGHV* mutational status and r-Binet stage were the only two variables independently associated with progression. In connection with this it is of note that r-Binet B patients also showed a higher rate of high-risk cytogenetics. These data are in line with the reported frequent association between lymphadenopathies and certain high-risk cytogenetic alterations such as del11q [22,23]. Importantly, our study revealed that the presence of abdominal lymphadenopathies negatively impacted on PFS. The low number of cases presenting thoracic lymphadenopathies precluded a statistically sound evaluation.

Binet A patients were subclassified into Rai low- and intermediate-risk cases. About 56% of low-risk patients showed lymphadenopathy and/or organomegaly using TB-CT scans and could be classified as r-Rai intermediate-risk patients. The latter comprised a significantly higher rate of cases with high CD38 and ZAP-70 expression and elevated β2-microglobulin levels. The higher β2-microglobulin levels likely reflects the CLL tumor burden [24]. Moreover, within the clinical Rai low-risk group, those reclassified as r-Rai intermediate-risk had a significantly shorter PFS. Also, r-Rai stage and *IGHV* mutational status remained independently associated with progression reaffirming the value of radiological data. Again in this subgroup the presence of abdominal lymphadenopathies was a predictor of progression, confirming data by Muntanola et al. [11].

We observed a high rate of cases (more than 40%) with lymphadenopathy and/or organomegaly at TB-CT scan within the cMBL group; these patients should not be considered as having true cMBL but SLL according to IWCLL guidelines [8]. In fact, the definition of SLL requires the presence of lymphadenopathy and/or splenomegaly (as defined by physical examination or CT scan) associated with $<5.0 \times 10^9$/l lymphocytes fulfilling the phenotypic features of CLL/SLL [8]. Likewise, the presence of lymphadenopathy and/or splenomegaly represent exclusion criteria for cMBL [8]. Accordingly, Rossi et al. reported that 15% of cMBLs progressed to SLLs, suggesting that some cMBLs are SLLs with a very low tumor burden at diagnosis [25]. Scarfò et al. found lymphadenopathy and/or hepato-/splenomegaly in only 6% of cMBL by radiologic evaluation, although the criteria utilized for defining lymphadenopathies were not clearly outlined in the study [26]. Overall, our and other data support the notion that TB-CT scan could correctly distinguish between cMBLs and SLLs. Unfortunately, the relatively low number of cases and events in our series does not allow to definitively establish whether r-cMBLs and r-SLLs have a different prognosis.

Finally, TB-CT scan allowed the early diagnosis of a concomitant neoplasm in 2 cases. It is known that patients with CLL have a high risk of developing a second cancer and an increased frequency of certain cancer types [27]. Nonetheless, there are no studies investigating the use of TB-CT scans in routine screening or follow-up to precociously detect cancer in CLL.

Overall, these results indicate that TB-CT scan, through a more accurate evaluation of tumor burden than clinical examination, allows the identification of a subset of cases with a more aggressive disease in early stage CLL patients. Moreover, in patients with $<5.0 \times 10^9$ B lymphocytes/l, TB-CT scan can differentiate between true cMBL and SLL cases. On the basis of our results it may be premature to recommend the routine use of TB-CT scan in all early stage CLL patients, as this recommendation should also consider obvious economic constraints. Nevertheless, our data provide groundwork for performing a larger study to verify the prognostic significance of TB-CT scan along with other new prognostic parameters in CLL patients.

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